

## **The use of preclinical models for malignant pleural mesothelioma**

Marie Shamseddin<sup>1,2</sup>, Joanna Obacz<sup>2</sup>, Mathew J Garnett<sup>1</sup>, Robert C. Rintoul<sup>3,4</sup>, Hayley E Francies<sup>1</sup>, Stefan J. Marciniak<sup>2,\*</sup>

<sup>1</sup>Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridgeshire CB10 1RQ, UK; <sup>2</sup>Cambridge Institute for Medical Research, Keith Peters Building, Cambridge Biomedical Campus, University of Cambridge, Cambridge CB2 0XY, UK; <sup>3</sup>Department of Oncology, University of Cambridge, Cambridge, UK; <sup>4</sup>Department of Thoracic Oncology, Royal Papworth Hospital NHS Foundation Trust, Cambridge CB2 0AY, UK

\*To whom correspondence should be addressed:

Stefan J. Marciniak

Cambridge Institute for Medical Research, Keith Peters Building, Cambridge Biomedical Campus, University of Cambridge, Cambridge CB2 0XY, UK

[sjm20@cam.ac.uk](mailto:sjm20@cam.ac.uk)

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## Abstract

Malignant pleural mesothelioma (MPM) is an aggressive cancer most commonly caused by prior exposure to asbestos. Median survival is 12-18 months, since surgery is ineffective and chemotherapy offers minimal benefit. Preclinical models that faithfully recapitulate the genomic and histopathological features of cancer are critical for the development of new treatments. The most commonly used models of MPM are two-dimensional (2D) cell lines established from primary tumours or pleural fluid. While these have provided some important insights into MPM biology, these cell models have significant limitations. In order to address some of these limitations spheroids and microfluidic chips have more recently been used to investigate the role of the 3D environment in MPM. Efforts have also been made to develop animal models of MPM, including asbestos-induced murine tumour models, MPM-prone genetically modified mice, and patient derived xenografts (PDX). Here we discuss the available *in vitro* and *in vivo* models of MPM and highlight their strengths and limitations. We discuss how newer technologies, such as the tumour-derived organoids, might allow us to address the limitations of existing models and aid in the identification of effective treatments for this challenging to treat disease.

## Introduction

Malignant pleural mesothelioma (MPM) is a relatively rare aggressive cancer[1]. It is most often caused through inhalation of asbestos fibres, although there is a long latency of more than 30 years between exposure and the development of disease[1]. Between 1994 to 2008, 92,253 mesothelioma deaths were reported to the WHO, with the majority of cases being from Europe, and age-adjusted mortality rates increased at 5.37% per year[1]. Following the ban on importation and use of asbestos in many developed nations it is predicted that MPM death rates will fall in these nations between 2020-2040[2–5]. For example, in the UK, where the use of all types of asbestos was fully banned in 1999[6], the mesothelioma incidence rate is projected to fall by 53% between 2014 and 2035[7]. Yet, although asbestos use is prohibited in many countries, the industrialisation of developing nations is fuelling the use of asbestos and subsequently the incidence of MPM is expected to rise in the coming decades[8].

MPM affects the mesothelial cells of the pleura and presents as three main histopathological subtypes (Table 1): polygonal “epithelioid” MPM is the most common subtype, representing 60% of cases; spindle-shaped “sarcomatoid” MPM accounts for 10% of cases; and biphasic mesothelioma, consisting of a mixture of epithelioid and sarcomatoid cells, accounts for the remaining 30%[9,10]. Mesothelial markers used to identify MPM include calretinin, Wilms’ tumour gene product (WT-1), mesothelin, cytokeratin (CK) 5/6, HBME-1 antigen, thrombomodulin and podoplanin (D2-40)[10]. However, being less differentiated, many

sarcomatoid MPM tumours only express CKs and a variable amount of calretinin, but gain expression of vimentin and smooth muscle markers[10]. The histopathological subtype as well as the stage of disease impacts overall survival with a diagnosis of epithelioid MPM being associated with the longest median survival (13.1 months) and sarcomatoid being associated with the worst survival (4 months)[11]. MPM also has great intra-tumour heterogeneity which has been studied using a deconvolution approach which uncovered that all MPM tumours are comprised of combinations of epithelioid-like and sarcomatoid-like components but the proportions of each are highly associated with prognosis[12].

First line treatments that have been proposed for MPM include chemotherapy, surgery, radiotherapy, separately or in combination. Surgical procedures such as extrapleural pneumonectomy (EPP) and pleurectomy/decortication have been proposed as curative or palliative approaches respectively[13–15]. However, the clinical outcome of the mesothelioma and radical surgery (MARS) trial suggested that EPP within tri-modal therapy has no benefit[16]. The benefit of open pleurectomy/decortication in addition to chemotherapy is currently under investigation in the MARS 2 trial[17]. The MesoVATS trial previously showed that a video-assisted thoracoscopic partial pleurectomy does not improve overall survival of patients[18]. At present, the benefits of surgery in MPM remains limited. Radiotherapy primarily has a role in palliation, but has been suggested as an adjuvant to surgery and chemotherapy in multimodality treatment[13,15]. Combination chemotherapy with pemetrexed and cisplatin has remained unchanged as the standard of care since 2003 following a phase III clinical trial demonstrating superiority of the combination over cisplatin alone (median survival 13.3 months vs. 10 months)[19]. In 2016, a phase III trial of the anti-VEGF recombinant antibody, bevacizumab, in combination with standard chemotherapy was shown to increase survival in comparison to chemotherapy alone (18.8 months vs. 16.1 months) across all MPM subtypes[20]. This regimen would not be suitable for patients with cardiovascular comorbidities[20] and currently is not available due to lack of FDA, EMA and NICE registration and universal reimbursement[21,22]. A recent phase III trial of combined PD-1 inhibition, using nivolumab, and anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) monoclonal antibody ipilimumab, revealed prolonged overall survival compared to the standard chemotherapy regimen[23]. This drug combination which has been recently approved by FDA[24] showed more benefit for patients with non-epithelioid tumours[23], while non-epithelioid subtypes of MPM were less responsive to chemotherapy or surgery[25,26].

Comprehensive analysis of MPM samples has revealed the genomic landscape of this disease. A key finding has been that the disease is dominated by loss of function mutations in a number of tumour suppressor genes including: 1. cyclin-dependent kinase inhibitor 2A (*CDKN2A*, alias for *INK4A/ARF*) with homozygous deletion in 45-49% of cases, encodes two

alternative reading frame proteins p14<sup>ARF</sup> and p16<sup>INK4A</sup> (involved in the p53 and retinoblastoma protein (RB1) pathways); 2. BRCA associated protein 1 (*BAP1*) mutations are observed in 22-57% of cases, encodes a tumour suppressor that regulates several processes including the cell cycle, cell death and the response to DNA damage; 3. neurofibromin 2 (*NF2*) is mutated in 19-50% of cases, encodes Merlin which is involved in the inactivation of the receptor-dependent mitogenic signalling pathway, inhibition of PI3K activity and regulation of the hippo pathway[27–40]. In addition, *TP53* mutations has been reported in 4-20% of cases[34,37,41,42]. *CDKN2B* (alias for *INK4B*), which is adjacent to *CDKN2A* and encodes p15<sup>INK4b</sup> also recurrently shows loss of copy number[33,34,43]. In the COSMIC database, recurrent mutations have also been found in a further set of genes including *PTEN* and *RB1*[34]. Comparisons of mutational profiles in the various MPM subtypes reveals common and exclusive mutations. Genetic alterations in *CDKN2A*, *BAP1* and *NF2* are common in all subtypes with higher frequency of *BAP1* and *NF2* in epithelioid and sarcomatoid respectively[34]. Other significantly mutated genes include *SETD2*, *LATS1*, *LATS2*, *CDKN2B*, *MST1* and *MTOR*[34]. While genetic alterations in *STK3*, *DDX3X*, *DDX51*, *SETD5*, *SF3B1* and *TRAF7* were exclusively detected in epithelioid and biphasic disease, genetic alterations in *SETDB1*, *TP53*, *TSC2*, *ULK2* and *SAV1* were found only in sarcomatoid and biphasic disease[34]. Notably, *TP53* mutations were associated with a lower survival rate[34]. Furthermore, classification of biphasic subtype to epithelioid like (biphasic-E) and sarcomatoid like (biphasic-S) based on RNA-sequencing, found genetic alterations in *STK3* and *DDX51* only in biphasic-E and *TSC2* only in biphasic-S[34] (Table 1).

Conversely, there are few activating oncogenic driver genes or protein-altering mutations in MPM compared with other solid tumours[34,37]. This unfortunately limits the number of available cancer-selective drug targets since existing drugs mostly target activating oncogenes. Nevertheless, studies of the fundamental biology of MPM have yielded potential novel targeted therapies that are currently being tested in clinical trials, including inhibitors of histone methyltransferase EZH2, focal adhesion kinase (FAK), mesothelin, PI3K, mTOR, PD-1 and PD-L1, and anti-angiogenic therapies such as VEGF inhibitors[44,45]. Unfortunately, many clinical trials in MPM have proved negative[44,45]. This may reflect the availability of drug resistance pathways to MPM tumours. Furthermore, the lack of biological biomarkers for responsiveness to most targeted therapies has precluded patient stratification and consequently clinical responses restricted to small patient cohorts might conceivably have been missed. However, careful patient selection might improve drug-responsiveness, as suggested by a recent phase IIa trial of a poly ADP ribose polymerase (PARP) inhibitor, rucaparib, which demonstrated efficacy in patients with *BAP1*/*BRCA1* deficient malignant mesothelioma[46].

The development of new therapeutic approaches for MPM requires a larger and more diverse panel of preclinical MPM models to recapitulate the patient population both genomically and histopathologically as well as the ability to model relevant drug response. Consequently, much effort has been invested in establishing 2D cell lines (cells grown as flat 2D cultures) from primary MPM tumours and pleural effusions[47,48]. More complex 3D *in vitro* models and a number of murine *in vivo* models have also been developed. Here, we discuss the available *in vitro* and *in vivo* preclinical MPM models, highlighting both their strengths and limitations, and the gaps that remain to be filled by improved models.

## **2D culture of human pleural mesothelioma cells**

Many human MPM cell lines have been established from tumour tissue and pleural effusions[48–70] with success rates ranging from 20% to 84%[54,64,68,70] (Supplementary Table 1). These cell lines represent a spectrum of MPM histopathological subtypes and many harbour the genetic aberrations commonly observed in MPM tumours, including inactivation of *NF2*, *CDKN2A* and *BAP1* genes[31,39,42,58,71,72]. However, most studies have not compared cell lines to the original tumour from which they were derived to determine how well they recapitulate the genomic and histopathological features. To date, such a comparison has only been conducted once and concordance of genomic alterations was found to be high between the tumour and early passage cell lines, although some single nucleotide variants (SNV) were found exclusively in the cell lines[49]. This could represent the expansion of a rare clone present in the tumour or acquisition of new SNVs during *in vitro* culture. Furthermore, in order to facilitate mesothelioma diagnosis, using a large panel of 61 mesothelioma cell lines from pleural effusions[73], a genome-wide analysis has been conducted. As a result, it was showed that the genes *COL3A1*, *SLPI*, *ITLN1*, and *CCL2* are expressed preferentially in MPM cells when compared to lung adenocarcinomas[73]. Accordingly, significantly higher levels of secreted *CCL2* were found in pleural effusions of MPM patients compared with pleural fluid from patients with other metastatic cancers or benign conditions[73].

The majority of available malignant cell lines can be passaged indefinitely. As such, genomic instability, including polyploidy[74], copy number alterations and the emergence of new aberrations[58,75] have been observed following long-term culture of MPM models. Moreover, kataegis, the accumulation of a large number of single nucleotide substitutions clustered in a single locus was reported in MPM cell cultures but not primary tumour tissue[49]. It has also been shown recently that newly derived primary mesothelioma cells display a significantly different transcriptome compared with established MPM cell lines[48]. Furthermore, long-term culturing of primary MPM cells can affect their response to drugs[76]. Therefore, as cell lines

adapt to 2D culture they lose many of the characteristics of the original tumour which can affect their use in research.

It has recently been demonstrated that cell lines represent only part of the sub-clonal diversity of primary tumours and that different sub-clones may be dominant in the mesothelioma tumours and derived cell lines[49]. In line with this, some groups reported the development of two morphologically distant MPM cell lines obtained from the same patient, with differential expression of genes and chromosomal aberrations[56,65]. Therefore, cell lines fail to recapitulate the complete tumour heterogeneity found in patient tumours.

Early passage, primary MPM cell lines have been exploited in a number of transcriptomic analyses, supporting the identification of putative diagnostic markers and treatment avenues. A recent transcriptomic analysis of a collection of primary MPM cultures, generated in the 1990s from patient samples obtained from French hospitals[77,78] led to the identification of distinct molecular subgroups of MPM with divergent prognoses. The observed differences could be attributed largely to the varying mutation profiles between subtypes as well as divergent deregulated pathways, including epithelial to mesenchymal transition and TGF $\beta$  signalling[79]. The molecular subtypes of MPM can also be predicted based on the differential expression of *PPL*, *UPK3B* and *TFP1* genes[79]. However, none of these studies have been translated to new diagnostic tools nor treatments for MPM.

Early passage MPM cell lines have been also used in drug sensitivity testing in a number of studies[58,76,80–82], demonstrating variability in drug response between individuals and indicates the importance of personalized medicine. Integration of drug sensitivity testing of eighty-one short-term primary mesothelioma cell cultures with gene expression data revealed three response groups corresponding to distinct gene signatures involving the FGF signalling pathway[80]. High-throughput drug sensitivity testing of a panel of commercial and primary early-passage mesothelioma cell lines identified a subgroup of MPM lines highly sensitive to FGFR inhibition as well as death receptor agonist tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), associated with BAP1 loss[81,82]. A phase Ib clinical trial of a FGF ligand trap in combination with pemetrexed/cisplatin chemotherapy appeared to show durable responses[83] but further validation is required before loss of BAP1 can be used as a biomarker for responsiveness to FGF/FGFR inhibitors or TRAIL.

Patient-derived mesothelioma cell lines therefore represent a simple model with which to study MPM biology and sensitivity to therapeutics. However, enthusiasm for their use in guiding personalised medicine must be tempered by important limitations: (i) their proclivity to adapt to 2D culture conditions thereby changing their phenotype, (ii) their failure to recapitulate

tumour heterogeneity, and (iii) the lack of immune and stromal cell interactions in culture conditions.

### **Animal models**

Animal models offer the ability to capture some of the complexity of the *in vivo* tumour environment that is known to contribute to disease progression and drug responsiveness. One major issue of animal models is the time and cost associated with them and subsequently making them unsuitable for large-scale phenotypic screens. Yet, such models are essential for drug testing and have substantially contributed to our understanding of MPM[84]. Several groups have generated MPM animal models including genetically modified mice, asbestos-induced murine models, as well as PDX models (Table 2).

### ***Genetically engineered mouse models***

MPM mouse models have been established through the alteration of genes known to be involved in human MPM. Mice with mesothelial-specific deletion of *Nf2*, *p53*, and *Ink4a/Arf* were generated using intra-thoracic injection of Adeno-Cre virus in homozygous and heterozygous conditional knockout (CKO) mice of *Nf2;p53*, *Nf2;Ink4a/Arf* and *Nf2;p53* carrying an inactive *Ink4a* allele (*Nf2;p53;Ink4a\**)[85]. Conditional *Nf2;Ink4a/Arf* mice demonstrated increased pleural invasion compared to conditional *Nf2;p53* mice[85]. Furthermore, homozygous CKO mice *Nf2;p53;Ink4a\** were highly malignant with invasion in 75% of tumours in both parietal and visceral pleura and had the worst survival with a median survival of around 11 weeks compared with the other homozygous CKO mice (around 19 and 31 weeks in *Nf2;p53*, *Nf2;Ink4a/Arf* respectively). These indicate that *Ink4a* loss leads to a more aggressive phenotype and poor clinical outcome of MPM[85]. Immunohistochemistry of the murine mesotheliomas identified the epithelioid MPM phenotype in the CKO mice *Nf2;p53* and *Nf2;Ink4a/Arf* mice, but not in *Nf2;p53;Ink4a\** mice. Sarcomatoid phenotypes were found in all CKO mice[85]. In this manner, by inactivating genes known to be mutated frequently in mesothelioma, a powerful model was generated that (i) yielded high incidence of spontaneous mesothelioma development and (ii) gave a short latency for tumour initiation. However, in humans with MPM, it is the epithelioid histological subtype that predominates, while most CKO mice developed sarcomatoid MPM. Only when CKO mice of *Nf2;p53;Ink4a/Arf* were exposed to asbestos using intrapleural injection, the tumours demonstrated predominantly epithelioid subtype[86]. Furthermore, in contrast to the reported rarity of *p53* mutations in human epithelioid MPM, CKO mice of *Nf2;p53* developed epithelioid MPM. This might reflect species differences with respect to the impact of these genes or relate to different oncogenic mechanisms, such as the prolonged effects of asbestos fibres in human patients versus acute onset of somatic mutations in mice[85].

Murine models have been generated by conditional knockout in the pleura, of various combinations of the genes most frequently altered in human MPM: *Bap1*, *Ink4a/Arf* and *Nf2*[87]. Homozygous deletion of each individual gene led to few or no malignant mesotheliomas of the pleura or peritoneum[87]. Mesotheliomas were observed following homozygous co-deletion of *Bap1;Nf2*, *Bap1;Ink4a/Arf* and *Nf2;Ink4a/Arf* in 17%, 22% and 63% of mice respectively, rising to 85% of mice in which all three genes were targeted[87]. The partial penetrance of MPM in all allelic combinations suggests partial redundancy amongst these tumour suppressors and that additional events are required to drive the phenotype. The median survival of *Nf2;Ink4a/Arf* mice was reproducibly around 27-35 weeks in different studies[85,87,88] (Table 2). The triple CKO mice had a shorter latency (12 to 16 weeks) compared to double CKO mice (21 to 40 weeks)[87,88]. Similarly, triple homozygous CKO *Bap1;Nf2;Ink4b/Ink4a/Arf* mice also have a short median survival of 12 weeks and display features similar to those of human MPMs including activation of the PI3K and MAPK pathways, substantial macrophage infiltration and the presence of a significant number of T cells, B cells and natural killer cells[88]. RNA sequencing revealed malignant mesotheliomas in the triple CKO mice compared to double CKO of *Nf2;Ink4a/Arf* were enriched in transcripts of genes controlled by polycomb repressive complex 2 (PRC2) including cancer related genes[87]. This argues that loss of BAP1, which forms part of a polycomb repressive deubiquitinase complex[27], contributes to tumour progression by loss of PRC2-mediated repression of oncogenic genes[87]. Consequently, these CKO mice are potential models to test therapeutics in these various genetic backgrounds. However, variability in the subtypes of tumours resulting from the same double and triple CKO mice in different studies may complicate interpretation of these results, with non-epithelioid subtypes in one study[87] and all three histological subtypes in others[85,88] (Table 2).

Given that PI3K signalling pathway appears hyper-activated in many human MPMs, when it should normally be antagonised by PTEN, a mouse model was generated by inactivation of *Pten* and *Tp53* in the mesothelium[89]. Both peritoneal and pleural malignant mesotheliomas developed in these double KO mice, while *Pten* inactivation alone was not sufficient for tumour development[89]. Most of the mesotheliomas were non-epithelioid, which is consistent with the low PTEN levels observed in human sarcomatoid tumours and with the association of *Tp53* mutations with non-epithelioid subtypes[89,90]. This model was associated with MEK/ERK and PI3K activation and, accordingly, inhibition of MEK and PI3K using Selumetinib and AZD8186 increased the survival of *Pten;p53* mice[90]. Although the *Pten;p53* mice provide a relevant preclinical model for mesothelioma with sarcomatoid features, the common somatic genetic alterations found in human MPM are not recapitulated in this model[89].

### ***Asbestos/Asbestos-like induced murine models***



Malignant mesothelioma can also be induced through murine exposure to asbestos fibres. Peritoneal mesothelioma is even rarer than MPM, perhaps because less asbestos reaches the human peritoneum or this might reflect a different mode of pathogenesis entirely. However, most asbestos-induced murine models have been developed by intraperitoneal injection of asbestos and subsequent investigation of extracted mesothelial cells from malignant ascites that formed within the model[42,91–97]. One reason for this experimental approach is that asbestos exposure by inhalation, which is more physiologically relevant to how MPM develops in humans, results in low pleural tumour burden in murine models[94]. Several studies demonstrated that inactivation of *Bap1*, *Nf2*, *Ink4a/Arf* and *Tp53* led to a higher incidence and rapid progression of malignant mesothelioma in comparison to the wildtype mice treated with asbestos[42,91–95,98].

Carbon nanotubes (CNT), which share some physical characteristics with asbestos, are increasingly used in medical and commercial applications[99]. Trans-tracheal intrapulmonary delivery of multiwalled CNTs in rats induces pleural and pericardial mesothelioma in 16% of rats between 24-27 months[100]. Similarly, in wildtype mice injection of long fibre CNTs into the pleural cavities causes pleural mesothelioma in 10-25% of animals compared with 9% of mice injected with asbestos[99]. Progression of murine mesothelioma lesions induced by CNT and asbestos were associated with increased cell proliferation and oxidative damage[99]. In this model, hypermethylation of the *Ink4a/Arf* locus before tumour development caused loss of p19<sup>Arf</sup> (homologue to human p14<sup>ARF</sup>) and p16<sup>Ink4a</sup> protein and led to deletion of *Arf* in end stage mesothelioma, recapitulating an epigenetic feature of human MPM[99]. The long latency of this model is both its strength and its main weakness. On one hand, it provides a valuable tool to study molecular events that occur during the latency period, such as the hypermethylation causing loss of p19<sup>Arf</sup>, but equally the time required to develop tumours (up to 20 months), make it unsuitable for drug testing. In addition to these issues, the rat model has yet to be characterised extensively at a molecular level.

### **Graft models**

Subcutaneous or intrathoracic injection of human MPM cell lines into mice are frequently used to study mesothelioma biology and treatment strategies[101–104]. Highly passaged human mesothelioma cell lines lack many characteristics of their original tumours and, importantly, generate little of the intra-tumour heterogeneity that is typical of the human disease. To date, there are few studies generating xenografts directly from human MPM tumour tissue or pleural fluid. In one study, subcutaneous xenografts were generated from early passage (below passage 5) primary mesothelioma cultures, derived from the pleural fluid of four patients with MPM and the ascitic fluid of one patient with peritoneal mesothelioma[105]. A larger bank of

xenograft models was established through subcutaneous implantation of fresh tumour material from 50 MPM patients with an engraftment rate of 40% and were subsequently passaged up to 5 generations[106]. These xenografts maintained the expression of markers of the primary cells including Mesothelin, Calretinin, WT1 and BAP1[105] and the histological subtype of epithelioid and sarcomatoid in the original tissue[106]. However, some of biphasic tumours gave rise to xenografts with either epithelioid or sarcomatoid histology only[106]. While most sarcomatoid and biphasic samples formed xenografts, only one third of epithelioid samples engrafted successfully and interestingly they were from patients with a poorer outcome[106]. This limits xenograft models largely to the study of more aggressive forms of MPM. Treatment of a subset of the models across different histological subtypes with cisplatin inhibited growth of 7 of 10 tested PDXs; however, pemetrexed, either alone or in combination with cisplatin, did not affect the growth of these xenografts owing to the metabolism of folate by NOD/SCID mice, which differs from that in humans[106]. Furthermore, engrafting the tumour from pleural mesothelium into subcutaneous tissue profoundly changes the tumour microenvironment. Finally, although PDX models are a valuable tool to assess the response of patients to specific treatments, the lack of an immune system limits their utility for testing immunotherapies.

To evaluate immunotherapies for mesothelioma, syngeneic murine models can be used[107–110]. In these models, murine cell lines are implanted into the immunocompetent host, for example subcutaneous or intraperitoneal injection of murine mesothelioma cells including: AB1, AB12, AB22 cells in BALB/c mice; AE17 cells in C57BL/6J mice; and F4-T2, F5-T1, M5-T1 cells in F344 Fischer rats[107–115]. Very few studies have used direct implantation of murine mesothelioma lines into the pleural space of mice[114]. In addition, all these murine mesothelioma cell lines were in fact established from peritoneal mesothelioma following intraperitoneal injection of asbestos[108,113,116]. While peritoneal and pleural mesothelioma might share some of the same biology, this has yet to be proved. In addition, similar to most available human MPM lines, murine mesothelioma cell lines suffer from having undergone clonal selection leading to adaptation to *in vitro* culture. Also, as with human cell lines, murine syngeneic tumour models fail to reproduce the intra-tumour and inter-patient heterogeneity seen in the clinic.

### 3D models

**Spheroids:** Several groups have cultured established mesothelioma cell lines or primary tumour tissue fragments in non- or low-adherent conditions leading to the formation of 3D structures called cancer spheroids. These have been used to study the biology of mesothelioma and test drugs[61,117–120](Figure 1a). Spheroid cultures recapitulate the

resistance of mesothelioma cells to apoptosis more effectively than 2D cultures[117,121–124]. This may reflect the very different transcriptional profiles that 2D versus spheroid culture conditions generate for the same lines[125,126]. Indeed, genes involved in apoptosis are downregulated in spheroids[125]. Although 3D cultures are likely to be an improvement upon classical 2D conditions, spheroids derived from cell lines will suffer many of the same limitations as 2D cultures, such as their lack of heterogeneity. By contrast, tumour-derived spheroids may overcome this and be maintained in culture for months. A single report of mesothelioma spheroids grown *in vitro* describes the retention of a proliferation rate similar to that of the original tumour for four weeks[120]. Unfortunately, there are currently no long-term spheroid models that permit expansion of cell number, which limits the range and number of experiments that can be conducted. Moreover, no studies have yet compared the genomic stability of MPM spheroids and their concordance with the original tumour.

***Tumour on a chip:*** Microfluidic chips offer another method to model the 3D geometrical shape and the dynamic microenvironment by providing a perfused system mimicking a vascularised tumour[127]. These devices have been used to develop a 3D model of MPM using either tumour biopsies directly from patients or spheroids derived from cell lines[127,128] (Figure 1b). Although this system has demonstrated potential to predict clinical response to chemotherapy, applicability to long-term culture or indeed systematic drug screening are likely to be challenging.

## Outlook

There has been a disappointing lack of progress in the treatments available for MPM patients and global incidence rates are set to increase. Many clinical trials have been conducted, often based on the results of experiments using simple preclinical models, but they have not translated from model to patient. This suggests that the current preclinical MPM models are not recapitulating human physiology sufficiently well and/or we lack enough models to capture the diversity of the disease. High-quality preclinical models are essential for the development of new treatments for this lethal cancer. Cell lines, spheroids and animal models each have their individual strengths and limitations, so no single model possesses every ideal feature. It is therefore essential that the choice of model is driven by the aim of a given study.

There has been great interest in the recent development of a 3D cell model technology called organoids[129] which have overcome many limitations of preclinical models. Organoids are grown in a 3D matrix such as basement membrane extract (BME) or Matrigel, enriched for laminins and collagens to resemble the basement membrane[129,130] (Figure 1c). Unlike spheroids, organoids can be propagated for long periods in culture using defined media[129] making them feasible to biobank and to be an accessible resource. There are now organoid

derivation protocols for many epithelial tissues and cancers including pancreas, colon, oesophagus, ovary, breast, prostate, endometrium, liver, and lung[129–138]. These studies have demonstrated how these models can be generated at relatively high success rates (70 to more than 90%) and importantly recapitulate genomic alterations and histopathological features of the tumour of origin as well as a degree of the subclonal architecture present in the tumour[129–138]. They are amenable to a number of experimental techniques including medium-throughput drug sensitivity testing[130,139] and importantly have been shown to recapitulate patient responses to chemotherapy as well as other anti-cancer agents in the setting of co-clinical trials or a prospective clinical study[140,141]. Furthermore, methods have been developed that enable the successful co-culture of organoids with other cell types found in the tumour microenvironment, such as immune and stromal cells, increasing the complexity of organoids culture to better recapitulate the tumour microenvironment[142–146]. Large scale efforts, such as Human Cancer Models Initiative, are now attempting to derive and characterise panels of cancer organoids that recapitulate the diversity of patient population and to make these models available to the community in order to identify new therapeutic strategies for a variety of cancer types. Unfortunately, to date organoid technology has not been applied to MPM. The development of MPM organoids to provide a strong tool with which to study MPM represents a significant hope for the future.

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Table 1: Characteristics of MPM subtypes

Subtype	Morphology	Markers	Prognosis (Months)	Genetic Alteration <sup>+</sup>
Epithelioid	Polygonal	Calretinin, WT-1, Mesothelin, CK5/6, Podoplanin, HBME-1 antigen, Thrombomodulin	13.1	<i>CDKN2A, BAP1, NF2, SETD2, LATS1, LATS2, CDKN2B, MST1, MTOR, STK3, DDX3X, DDX51, SETD5, SF3B1 and TRAF7.</i>
Sarcomatoid	Spindle-shaped	Mostly express only CKs, Calretinin, vimentin and Smooth muscle	4	<i>CDKN2A, BAP1, NF2, SETD2, LATS1, LATS2, CDKN2B, MST1, MTOR, SETDB1, TP53, TSC2, ULK2 and SAV1.</i>
Biphasic-E	Mixed	Mixed	8.4	<i>CDKN2A, BAP1, NF2, SETD2, LATS1, LATS2, CDKN2B, MST1, MTOR, TSC1, STK3, DDX3X, DDX51, SETD5, SF3B1, TRAF7, SETDB1, TP53, ULK2 and SAV1</i>
Biphasic-S				<i>CDKN2A, BAP1, NF2, SETD2, LATS1, LATS2, CDKN2B, MST1, MTOR, DDX3X, SETD5, SF3B1, TRAF7, SETDB1, TP53, TSC1, TSC2, ULK2 and SAV1</i>
Ref	[9,10]	[10]	[11]	[34]

<sup>+</sup>Subtypes for genetic alterations refer to the molecular classification based on RNA-sequencing data

Table 2. List of Animal Models of MPM

Animal Model	Method (if applicable)	Altered Genes (if applicable)	Number of MPM patients (if applicable)	Subtypes of MPM (if applicable)	Tumour rate	Median survival (weeks)	Ref
Asbestos/Asbestos like-induced	Intraleural injection of carbon nanotube and asbestos	-	-	NA	9.4%-25%	48-80	[99]
	trans-tracheal intrapulmonary spraying of multiwalled CNT <sup>+</sup>	-	-	NA	15.8%	70-109	[100]
Conditional mouse model	Intrathoracic injection with Adeno-Cre virus <sup>++</sup>	<i>Nf2;p53 (hom, hom)</i>	-	All subtypes	85.5%	19.3	[85]
		<i>Nf2;p53 (het, hom)</i>	-	All subtypes	61.8%	30.7	
		<i>Nf2;p53 (hom, het)</i>	-	Epithelioid and sarcomatoid	40%	86.4	
		<i>Nf2;lnk4a/Arf (hom, hom)</i>	-	All subtypes, Predominantly sarcomatoid and biphasic	82.5%	31.4	
		<i>Nf2;lnk4a/Arf (het, hom)</i>	-	All subtypes	34.1%	58.6	
		<i>Nf2;lnk4a/Arf (hom, het)</i>	-	All subtypes	34.6%	70.7	
		<i>Nf2;p53;lnk4a* (hom, hom, hom)</i>	-	Sarcomatoid and biphasic	100%	11.4	
		<i>Nf2;p53;lnk4a* (hom, hom, het)</i>	-	Sarcomatoid and biphasic	93.8%	NA	
	Intraleural injection with Adeno-Cre virus	<i>Nf2; lnk4a/Arf (hom, hom)</i>	-	Predominantly sarcomatoid and some biphasic[87], All subtypes[88]	63%[87]	27[87], 34.8[88]	[87,88]
		<i>Bap1;Nf2 (hom, hom)</i>	-	Predominantly biphasic and some sarcomatoid	17%	21	[87]

		<i>Bap1; Ink4a/Arf (hom, hom)</i>	-	Predominantly sarcomatoid and some biphasic	22%	40	
		<i>Bap1;Nf2;Ink4a/Arf (hom, hom, hom)</i>	-	Predominantly sarcomatoid and some biphasic[87], All subtypes[88]	85%[87]	12[87], 16.3[88]	[87,88]
		<i>NF2; Ink4b/Ink4a/Arf (hom, hom)</i>	-	NA	75%	27.1	[88]
		<i>Bap1; NF2; Ink4b/Ink4a/Arf (hom, hom, hom)</i>	-	NA	NA	12.1	
		<i>Bap1; NF2; Ink4b/Ink4a/Arf (het, hom, hom)</i>	-	NA	NA	20	
	Intrathoracic injection with Adeno-Cre virus	<i>Pten;Tp53</i>	-	Sarcomatoid [89,90] and biphasic[89]	56%[89]	19.3[89]	[89,90]
Conditional mouse model exposed to asbestos	Intrapleural injection with Adeno-Cre virus and Intrapleural injection of asbestos	<i>Nf2;p53; Ink4a/Arf</i>	-	Predominantly epithelioid (>90%) and sarcomatoid	NA	21 (post Cre-induction wo asbestos ) and 12 (post Cre induction with asbestos )	[86]
Patient-derived xenograft	Subcutaneous	-	50	All subtypes	-	-	[106]
	Subcutaneous	-	4	Epithelioid	-	-	[105]

\*The numbers indicate the total of pleural and pericardial mesothelioma.

<sup>++</sup>The numbers indicate thoracic tumours including MPM, rhabdomyosarcomas, and schwannomas  
hom: homozygous; het: heterozygous.



Figure 1. Current and potential future 3D models of MPM. a) Spheroids are obtained by culturing the cell lines or dissected primary tissues as small as 1 mm on a non or low-adherent plate. b) Microfluidic chips are implemented to model MPM using cell line derived spheroids or digested tumour tissue from patients. Arrows shows the medium flow. C) Potential future 3D model of organoids is obtained using defined medium and a 3D matrix.

## References:

- 1 Delgermaa V, Takahashi K, Park EK, *et al.* Les décès mondiaux par mésothéliome rapportés à l'Organisation mondiale de la Santé entre 1994 et 2008. *Bull World Health Organ* 2011;**89**:716–24. doi:10.2471/BLT.11.086678
- 2 Kwak KM, Paek D, Hwang S sik, *et al.* Estimated future incidence of malignant mesothelioma in South Korea: Projection from 2014 to 2033. *PLoS One* 2017;**12**:1–14. doi:10.1371/journal.pone.0183404
- 3 Oddone E, Bollon J, Nava CR, *et al.* Predictions of mortality from pleural mesothelioma in Italy after the ban of asbestos use. *Int J Environ Res Public Health* 2020;**17**:1–11. doi:10.3390/ijerph17020607
- 4 Price B, Ware A. Time trend of mesothelioma incidence in the United States and projection of future cases: An update based on SEER data for 1973 through 2005 U.S. Mesothelioma Incidence Time Trend and Projections B. Price and A. Ware. *Crit Rev Toxicol* 2009;**39**:576–88. doi:10.1080/10408440903044928
- 5 Bianchi C, Bianchi T. Global mesothelioma epidemic: Trend and features. *Indian J Occup Environ Med* 2014;**18**:82–8. doi:10.4103/0019-5278.146897
- 6 The Asbestos (Prohibitions) (Amendment) Regulations 1999.
- 7 Mesothelioma incidence statistics | Cancer Research UK.  
<https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/mesothelioma/incidence#heading=Four> (accessed 14 May 2020).
- 8 Jiang Z, Chen T, Chen J, *et al.* Hand-spinning chrysotile exposure and risk of malignant mesothelioma: A case–control study in Southeastern China. *Int J Cancer* 2018;**142**:514–23. doi:10.1002/ijc.31077
- 9 Husain AN, Colby T V., Ordóñez NG, *et al.* Guidelines for pathologic diagnosis of Malignant Mesothelioma: 2017 Update of the consensus statement from the International Mesothelioma Interest Group. *Arch Pathol Lab Med* 2018;**142**:89–108. doi:10.5858/arpa.2017-0124-RA
- 10 van Zandwijk N, Clarke C, Henderson D, *et al.* Guidelines for the diagnosis and treatment of malignant pleural mesothelioma. *J Thorac Dis* 2013;**5**:E254. doi:10.3978/j.issn.2072-1439.2013.11.28
- 11 Beckett P, Edwards J, Fennell D, *et al.* Demographics, management and survival of patients with malignant pleural mesothelioma in the National Lung Cancer Audit in England and Wales. *Lung Cancer* 2015;**88**:344–8. doi:10.1016/j.lungcan.2015.03.005
- 12 Blum Y, Meiller C, Quetel L, *et al.* Dissecting heterogeneity in malignant pleural mesothelioma through histo-molecular gradients for clinical applications. *Nat Commun* 2019;**10**:1–12. doi:10.1038/s41467-019-09307-6
- 13 Sayan M, Eren MF, Gupta A, *et al.* Current treatment strategies in malignant pleural mesothelioma with a treatment algorithm. *Adv Respir Med* 2019;**87**:289–97. doi:10.5603/ARM.2019.0051
- 14 Treasure PT, Sedrakyan A. Pleural mesothelioma: Little evidence, still time to do trials. *Lancet* 2004;**364**:1183–5. doi:10.1016/S0140-6736(04)17108-0
- 15 Bibby AC, Tsim S, Kanellakis N, *et al.* Malignant pleural mesothelioma: An update on investigation, diagnosis and treatment. *Eur Respir Rev* 2016;**25**:472–86.

doi:10.1183/16000617.0063-2016

- 16 Treasure T, Lang-Lazdunski L, Waller D, *et al.* Extra-pleural pneumonectomy versus no extra-pleural pneumonectomy for patients with malignant pleural mesothelioma: clinical outcomes of the Mesothelioma and Radical Surgery (MARS) randomised feasibility study. *Lancet Oncol* 2011;**12**:763–72. doi:10.1016/S1470-2045(11)70149-8
- 17 Lim E, Darlison L, Edwards J, *et al.* Mesothelioma and Radical Surgery 2 (MARS 2): protocol for a multicentre randomised trial comparing (extended) pleurectomy decortication versus no (extended) pleurectomy decortication for patients with malignant pleural mesothelioma. *BMJ Open* 2020;**10**:e038892. doi:10.1136/bmjopen-2020-038892
- 18 Rintoul RC, Ritchie AJ, Edwards JG, *et al.* Efficacy and cost of video-assisted thoracoscopic partial pleurectomy versus talc pleurodesis in patients with malignant pleural mesothelioma (MesoVATS): An open-label, randomised, controlled trial. *Lancet* 2014;**384**:1118–27. doi:10.1016/S0140-6736(14)60418-9
- 19 Vogelzang NJ, Rusthoven JJ, Symanowski J, *et al.* Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. *J Clin Oncol* 2003;**21**:2636–44. doi:10.1200/JCO.2003.11.136
- 20 Zalcman G, Mazieres J, Margery J, *et al.* Bevacizumab for newly diagnosed pleural mesothelioma in the Mesothelioma Avastin Cisplatin Pemetrexed Study (MAPS): A randomised, controlled, open-label, phase 3 trial. *Lancet* 2016;**387**:1405–14. doi:10.1016/S0140-6736(15)01238-6
- 21 Nowak AK, Brosseau S, Cook A, *et al.* Antiangiogenic Strategies in Mesothelioma. *Front Oncol* 2020;**10**:1–13. doi:10.3389/fonc.2020.00126
- 22 Project information | Bevacizumab for untreated malignant pleural mesothelioma (ID1183) | Guidance | NICE.
- 23 Bristol Myers Squibb - Opdivo® (nivolumab) Plus Yervoy® (ipilimumab) Demonstrates Durable Survival Benefit vs. Chemotherapy in Patients with Previously Untreated Malignant Pleural Mesothelioma. <https://news.bms.com/news/details/2020/Opdivo-nivolumab-Plus-Yervoy-ipilimumab-Demonstrates-Durable-Survival-Benefit-vs.-Chemotherapy-in-Patients-with-Previously-Untreated-Malignant-Pleural-Mesothelioma/default.aspx> (accessed 23 Sep 2020).
- 24 FDA Approves Drug Combination for Treating Mesothelioma | FDA. <https://www.fda.gov/news-events/press-announcements/fda-approves-drug-combination-treating-mesothelioma> (accessed 25 Oct 2020).
- 25 Mansfield AS, Symanowski JT, Peikert T. Systematic review of response rates of sarcomatoid malignant pleural mesotheliomas in clinical trials. *Lung Cancer* 2014;**86**:133–6. doi:10.1016/j.lungcan.2014.08.017
- 26 Meyerhoff RR, Yang CFJ, Speicher PJ, *et al.* Impact of mesothelioma histologic subtype on outcomes in the Surveillance, Epidemiology, and End Results database. *J Surg Res* 2015;**196**:23–32. doi:10.1016/j.jss.2015.01.043
- 27 Carbone M, Yang H, Pass HI, *et al.* BAP1 and cancer. *Nat Rev Cancer* 2013;**13**:153–9. doi:10.1038/nrc3459
- 28 Sherr CJ. The INK4a/ARF network in tumour suppression. *Nat Rev Mol Cell Biol* 2001;**2**:731–7. doi:10.1038/35096061
- 29 Nasu M, Emi M, Pastorino S, *et al.* High incidence of somatic BAP1 alterations in sporadic malignant mesothelioma. *J Thorac Oncol* 2015;**10**:565–76.

doi:10.1097/JTO.0000000000000471

- 30 Sekido Y, Bader S, Gazdar AF, *et al.* Neurofibromatosis Type 2 (NF2) Gene Is Somatic Mutated in Mesothelioma but not in Lung Cancer. *Cancer Res* 1995;**55**:1227–31.
- 31 Bott M, Brevet M, Taylor BS, *et al.* The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nat Genet* 2011;**43**:668–72. doi:10.1038/ng.855
- 32 Testa JR, Cheung M, Pei J, *et al.* Germline BAP1 mutations predispose to malignant mesothelioma. *Nat Genet* 2011;**43**:1022–6. doi:10.1038/ng.912
- 33 Guo G, Chmielecki J, Goparaju C, *et al.* Whole-exome sequencing reveals frequent genetic alterations in BAP1, NF2, CDKN2A, and CUL1 in malignant pleural mesothelioma. *Cancer Res* 2015;**75**:264–9. doi:10.1158/0008-5472.CAN-14-1008
- 34 Bueno R, Stawiski EW, Goldstein LD, *et al.* Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. *Nat Genet* 2016;**48**:407–16. doi:10.1038/ng.3520
- 35 Kato S, Tomson BN, Buys TPH, *et al.* Genomic landscape of malignant mesotheliomas. *Mol Cancer Ther* 2016;**15**:2498–507. doi:10.1158/1535-7163.MCT-16-0229
- 36 Ugurluer G, Chang K, Gamez ME, *et al.* Genome-based mutational analysis by next generation sequencing in patients with malignant pleural and peritoneal mesothelioma. *Anticancer Res* 2016;**36**:2331–8.
- 37 Hmeljak J, Sanchez-Vega F, Hoadley KA, *et al.* Integrative molecular characterization of malignant pleural mesothelioma. *Cancer Discov* 2018;**8**:1549–65. doi:10.1158/2159-8290.CD-18-0804
- 38 Petrilli AM, Fernández-Valle C. Role of Merlin/NF2 inactivation in tumor biology. *Oncogene* 2016;**35**:537–48. doi:10.1038/onc.2015.125
- 39 Cheng JQ, Jhanwar SC, Klein WM, *et al.* p16 alterations and deletion mapping of 9p21-p22 in malignant mesothelioma. *Cancer Res* 1994;**54**:5547–51.
- 40 Bianchi AB, Mitsunaga SI, Cheng JQ, *et al.* High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas. *Proc Natl Acad Sci U S A* 1995;**92**:10854–8. doi:10.1073/pnas.92.24.10854
- 41 Kang HC, Kim HK, Lee S, *et al.* Abstract 108: Whole exome and targeted deep sequencing identify genome-wide allelic loss and frequent SETDB1 mutations in malignant pleural mesotheliomas. 2016;**7**:108–108. doi:10.1158/1538-7445.am2016-108
- 42 Altomare DA, Vaslet CA, Skele KL, *et al.* A mouse model recapitulating molecular features of human mesothelioma. *Cancer Res* 2005;**65**:8090–5. doi:10.1158/0008-5472.CAN-05-2312
- 43 Xiao S, Li D, Vijg J, *et al.* Codeletion of p15 and p16 in primary malignant mesothelioma. *Oncogene* 1995;**11**:511–5.
- 44 Yap TA, Aerts JG, Popat S, *et al.* Novel insights into mesothelioma biology and implications for therapy. *Nat Rev Cancer* 2017;**17**:475–88. doi:10.1038/nrc.2017.42
- 45 Bronte G, Incorvaia L, Rizzo S, *et al.* The resistance related to targeted therapy in malignant pleural mesothelioma: Why has not the target been hit yet? *Crit Rev Oncol Hematol* 2016;**107**:20–32. doi:10.1016/j.critrevonc.2016.08.011

- 46 Fennell DA, Darlison L, Wells-Jordan P, *et al.* MiST1: A phase IIa trial of rucaparib in patients harbouring BAP1/BRCA1 deficient relapsed malignant mesothelioma. *J Clin Oncol* 2020;**38**:9057–9057. doi:10.1200/jco.2020.38.15\_suppl.9057
- 47 Rintoul RC, Rassl DM, Gittins J, *et al.* Mesobank UK: An international mesothelioma bioresource. *Thorax* 2016;**71**:380–2. doi:10.1136/thoraxjnl-2015-207496
- 48 Chernova T, Sun XM, Powley IR, *et al.* Molecular profiling reveals primary mesothelioma cell lines recapitulate human disease. *Cell Death Differ* 2016;**23**:1152–64. doi:10.1038/cdd.2015.165
- 49 Oey H, Daniels M, Relan V, *et al.* Whole-genome sequencing of human malignant mesothelioma tumours and cell lines. *Carcinogenesis* 2019;**40**:724–34. doi:10.1093/carcin/bgz066
- 50 Pass HI, Stevens EJ, Oie H, *et al.* Characteristics of nine newly derived mesothelioma cell lines. *Ann Thorac Surg* 1995;**59**:835–44. doi:10.1016/0003-4975(95)00045-m
- 51 Schmitter D, Lauber B, Fagg B, *et al.* Hematopoietic growth factors secreted by seven human pleural mesothelioma cell lines: interleukin-6 production as a common feature. *Int J Cancer* 1992;**51**:296–301. doi:10.1002/ijc.2910510220
- 52 Manning LS, Whitaker D, Murch AR, *et al.* Establishment and characterization of five human malignant mesothelioma cell lines derived from pleural effusions. *Int J Cancer* 1991;**47**:285–90. doi:10.1002/ijc.2910470219
- 53 Pelin-Enlund K, Husgafvel-Pursiainen K, Tammilehto L, *et al.* Asbestos-related malignant mesothelioma: growth, cytology, tumorigenicity and consistent chromosome findings in cell lines from five patients. *Carcinogenesis* 1990;**11**:673–81. doi:10.1093/carcin/11.4.673
- 54 Versnel MA, Bouts MJ, Hoogsteden HC, *et al.* Establishment of human malignant mesothelioma cell lines. *Int J Cancer* 1989;**44**:256–60. doi:10.1002/ijc.2910440212
- 55 Kobayashi M, Takeuchi T, Ohtsuki Y. Establishment of three novel human malignant pleural mesothelioma cell lines: Morphological and cytogenetical studies and EGFR mutation status. *Anticancer Res* 2008;**28**:197–208.
- 56 Schulten HJ, Perske C, Thelen P, *et al.* Establishment and characterization of two distinct malignant mesothelioma cell lines with common clonal origin. *Cancer Genet Cytogenet* 2007;**176**:35–47. doi:10.1016/j.cancergencyto.2007.03.005
- 57 Goudarzi H, Hida Y, Takano H, *et al.* Hypoxia affects in vitro growth of newly established cell lines from patients with malignant pleural mesothelioma. *Biomed Res* 2013;**34**:13–21. doi:10.2220/biomedres.34.13
- 58 Kanellakis NI, Asciak R, Hamid MA, *et al.* Patient-derived malignant pleural mesothelioma cell cultures: a tool to advance biomarker-driven treatments. *Thorax* 2020;:thoraxjnl-2020-215027. doi:10.1136/thoraxjnl-2020-215027
- 59 Schelch K, Hoda MA, Klikovits T, *et al.* Fibroblast growth factor receptor inhibition is active against mesothelioma and synergizes with radio- and chemotherapy. *Am J Respir Crit Care Med* 2014;**190**:763–72. doi:10.1164/rccm.201404-0658OC
- 60 Kopecka J, Salaroglio IC, Righi L, *et al.* Loss of C/EBP-beta LIP drives cisplatin resistance in malignant pleural mesothelioma. *Lung Cancer* 2018;**120**:34–45. doi:10.1016/j.lungcan.2018.03.022
- 61 Laszlo V, Valko Z, Ozsvar J, *et al.* The FAK inhibitor BI 853520 inhibits spheroid formation and orthotopic tumor growth in malignant pleural mesothelioma. *J Mol Med*

2019;**97**:231–42. doi:10.1007/s00109-018-1725-7

- 62 Cincinelli R, Musso L, Artali R, *et al.* Hybrid topoisomerase I and HDAC inhibitors as dual action anticancer agents. *PLoS One* 2018;**13**:e0205018. doi:10.1371/journal.pone.0205018
- 63 Bogden A, Griffin TW. Characterization of a human malignant mesothelioma cell line (H-MESO-1): A biphasic solid and ascitic tumor model. *Cancer Res* 1987;**47**:3199–205.
- 64 Relan V, Morrison L, Parsonson K, *et al.* Phenotypes and Karyotypes of Human Malignant Mesothelioma Cell Lines. *PLoS One* 2013;**8**. doi:10.1371/journal.pone.0058132
- 65 Usami N, Fukui T, Kondo M, *et al.* Establishment and characterization of four malignant pleural mesothelioma cell lines from Japanese patients. *Cancer Sci* 2006;**97**:387–94. doi:10.1111/j.1349-7006.2006.00184.x
- 66 Kawaguchi K, Murakami H, Taniguchi T, *et al.* Combined inhibition of MET and EGFR suppresses proliferation of malignant mesothelioma cells. *Carcinogenesis* 2009;**30**:1097–105. doi:10.1093/carcin/bgp097
- 67 Orecchia S, Schillaci F, Salvio M, *et al.* Aberrant E-cadherin and gamma-catenin expression in malignant mesothelioma and its diagnostic and biological relevance. *Lung Cancer* 2004;**45 Suppl 1**:S37-43. doi:10.1016/j.lungcan.2004.04.027
- 68 Philippeaux MM, Pache JC, Dahoun S, *et al.* Establishment of permanent cell lines purified from human mesothelioma: morphological aspects, new marker expression and karyotypic analysis. *Histochem Cell Biol* 2004;**122**:249–60. doi:10.1007/s00418-004-0701-1
- 69 Ishiwata I, Ishiwata C, Anzo M, *et al.* Establishment and characterization of a human malignant mesothelioma cell line (HMMME). *Hum Cell* 2003;**16**:231–9. doi:10.1111/j.1749-0774.2003.tb00157.x
- 70 Orengo AM, Spoletini L, Procopio A, *et al.* Establishment of four new mesothelioma cell lines: characterization by ultrastructural and immunophenotypic analysis. *Eur Respir J* 1999;**13**:527–34. doi:10.1183/09031936.99.13352799
- 71 Thurneysen C, Opitz I, Kurtz S, *et al.* Functional inactivation of NF2/merlin in human mesothelioma. *Lung Cancer* 2009;**64**:140–7. doi:10.1016/j.lungcan.2008.08.014
- 72 Illei PB, Rusch VW, Zakowski MF, *et al.* Homozygous deletion of CDKN2A and codeletion of the methylthioadenosine phosphorylase gene in the majority of pleural mesotheliomas. *Clin Cancer Res* 2003;**9**:2108–13.
- 73 Gueugnon F, Leclercq S, Blanquart C, *et al.* Identification of novel markers for the diagnosis of malignant pleural mesothelioma. *Am J Pathol* 2011;**178**:1033–42. doi:10.1016/j.ajpath.2010.12.014
- 74 Relan V, Morrison L, Parsonson K, *et al.* Phenotypes and karyotypes of human malignant mesothelioma cell lines. *PLoS One* 2013;**8**:e58132. doi:10.1371/journal.pone.0058132
- 75 Zanazzi C, Hersmus R, Veltman IM, *et al.* Gene expression profiling and gene copy-number changes in malignant mesothelioma cell lines. *Genes Chromosom Cancer* 2007;**46**:895–908. doi:10.1002/gcc.20475
- 76 Szulkin A, Ötvös R, Hillerdal CO, *et al.* Characterization and drug sensitivity profiling of primary malignant mesothelioma cells from pleural effusions. *BMC Cancer*

2014;**14**:1–13. doi:10.1186/1471-2407-14-709

- 77 Zeng L, Buard A, Monnet I, *et al.* In vitro effects of recombinant human interferon gamma on human mesothelioma cell lines. *Int J Cancer* 1993;**55**:515–20. doi:10.1002/ijc.2910550331
- 78 Zeng L, Fleury-Feith J, Monnet I, *et al.* Immunocytochemical characterization of cell lines from human malignant mesothelioma: characterization of human mesothelioma cell lines by immunocytochemistry with a panel of monoclonal antibodies. *Hum Pathol* 1994;**25**:227–34. doi:10.1016/0046-8177(94)90192-9
- 79 de Reynies A, Jaurand MC, Renier A, *et al.* Molecular classification of malignant pleural mesothelioma: identification of a poor prognosis subgroup linked to the epithelial-to-mesenchymal transition. *Clin Cancer Res* 2014;**20**:1323–34. doi:10.1158/1078-0432.CCR-13-2429
- 80 Schunselaar LM, Quispel-Janssen J, Kim Y, *et al.* Chemical Profiling of Primary Mesothelioma Cultures Defines Subtypes with Different Expression Profiles and Clinical Responses. *Clin Cancer Res* 2018;**24**:1761–70. doi:10.1158/1078-0432.CCR-17-1345
- 81 Kolluri KK, Alifrangis C, Kumar N, *et al.* Loss of functional BAP1 augments sensitivity to TRAIL in cancer cells. *Elife* 2018;**7**:1–23. doi:10.7554/eLife.30224
- 82 Quispel-Janssen JM, Badhai J, Schunselaar L, *et al.* Comprehensive Pharmacogenomic Profiling of Malignant Pleural Mesothelioma Identifies a Subgroup Sensitive to FGFR Inhibition. *Clin Cancer Res* 2018;**24**:84–94. doi:10.1158/1078-0432.CCR-17-1172
- 83 van Brummelen EMJ, Levchenko E, Dómine M, *et al.* A phase Ib study of GSK3052230, an FGF ligand trap in combination with pemetrexed and cisplatin in patients with malignant pleural mesothelioma. *Invest New Drugs* 2020;**38**:457–67. doi:10.1007/s10637-019-00783-7
- 84 Testa JR, Berns A. Preclinical Models of Malignant Mesothelioma. *Front Oncol* 2020;**10**:1–7. doi:10.3389/fonc.2020.00101
- 85 Jongsma J, van Montfort E, Vooijs M, *et al.* A Conditional Mouse Model for Malignant Mesothelioma. *Cancer Cell* 2008;**13**:261–71. doi:10.1016/j.ccr.2008.01.030
- 86 Farahmand P, Gyuraszova K, Rooney C, *et al.* Asbestos accelerates disease onset in a genetic model of Malignant Pleural Mesothelioma. *bioRxiv* 2020;:2020.06.16.154815. doi:10.1101/2020.06.16.154815
- 87 Kukuyan AM, Sementino E, Kadariya Y, *et al.* Inactivation of BAP1 cooperates with losses of NF2 and CDKN2A to drive the development of pleural malignant mesothelioma in conditional mouse models. *Cancer Res* 2019;**79**:4113–23. doi:10.1158/0008-5472.CAN-18-4093
- 88 Badhai J, Pandey GK, Song J, *et al.* Combined deletion of Bap1 , Nf2 , and Cdkn2ab causes rapid onset of malignant mesothelioma in mice. 2020;**217**.
- 89 Sementino E, Menges CW, Kadariya Y, *et al.* Inactivation of Tp53 and Pten drives rapid development of pleural and peritoneal malignant mesotheliomas. *J Cell Physiol* 2018;**233**:8952–61. doi:10.1002/jcp.26830
- 90 Marques M, Tranchant R, Risa-Ebrí B, *et al.* Combined MEK and PI3K/p110 $\beta$  inhibition as a novel targeted therapy for malignant mesothelioma displaying sarcomatoid features. *Cancer Res* 2020;**80**:843–56. doi:10.1158/0008-5472.CAN-19-1633

- 91 Napolitano A, Pellegrini L, Dey A, *et al.* Minimal asbestos exposure in germline BAP1 heterozygous mice is associated with deregulated inflammatory response and increased risk of mesothelioma. *Oncogene* 2016;**35**:1996–2002. doi:10.1038/onc.2015.243
- 92 Xu J, Kadariya Y, Cheung M, *et al.* Germline mutation of Bap1 accelerates development of asbestos-induced malignant mesothelioma. *Cancer Res* 2014;**74**:4388–97. doi:10.1158/0008-5472.CAN-14-1328
- 93 Altomare DA, Menges CW, Xu J, *et al.* Losses of both products of the cdkn2a/arf locus contribute to asbestos-induced mesothelioma development and cooperate to accelerate tumorigenesis. *PLoS One* 2011;**6**:6–11. doi:10.1371/journal.pone.0018828
- 94 Fleury-Feith J, Lecomte C, Renier A, *et al.* Hemizyosity of Nf2 is associated with increased susceptibility to asbestos-induced peritoneal tumours. *Oncogene* 2003;**22**:3799–805. doi:10.1038/sj.onc.1206593
- 95 Vaslet CA, Messier NJ, Kane AB. Accelerated progression of asbestos-induced mesotheliomas in heterozygous p53+/- mice. *Toxicol Sci* 2002;**68**:331–8. doi:10.1093/toxsci/68.2.331
- 96 Robinson C, Van Bruggen I, Segal A, *et al.* A novel SV40 TAg transgenic model of asbestos-induced mesothelioma: Malignant transformation is dose dependent. *Cancer Res* 2006;**66**:10786–94. doi:10.1158/0008-5472.CAN-05-4668
- 97 Sneddon S, Patch AM, Dick IM, *et al.* Whole exome sequencing of an asbestos-induced wild-type murine model of malignant mesothelioma. *BMC Cancer* 2017;**17**:1–10. doi:10.1186/s12885-017-3382-6
- 98 Kadariya Y, Cheung M, Xu J, *et al.* Bap1 is a bona fide tumor suppressor: Genetic evidence from mouse models carrying heterozygous germline Bap1 mutations. *Cancer Res* 2016;**76**:2836–44. doi:10.1158/0008-5472.CAN-15-3371
- 99 Chernova T, Murphy FA, Galavotti S, *et al.* Long-Fiber Carbon Nanotubes Replicate Asbestos-Induced Mesothelioma with Disruption of the Tumor Suppressor Gene Cdkn2a (Ink4a/Arf). *Curr Biol* 2017;**27**:3302-3314.e6. doi:10.1016/j.cub.2017.09.007
- 100 Suzui M, Futakuchi M, Fukamachi K, *et al.* Multiwalled carbon nanotubes intratracheally instilled into the rat lung induce development of pleural malignant mesothelioma and lung tumors. *Cancer Sci* 2016;**107**:924–35. doi:10.1111/cas.12954
- 101 Colin DJ, Cottet-Dumoulin D, Faivre A, *et al.* Experimental model of human malignant mesothelioma in athymic mice. *Int J Mol Sci* 2018;**19**. doi:10.3390/ijms19071881
- 102 Watanabe Y, Kojima T, Kagawa S, *et al.* A novel translational approach for human malignant pleural mesothelioma: Heparanase-assisted dual virotherapy. *Oncogene* 2010;**29**:1145–54. doi:10.1038/onc.2009.415
- 103 Di Martino S, Amoreo CA, Nuvoli B, *et al.* HSP90 inhibition alters the chemotherapy-driven rearrangement of the oncogenic secretome. *Oncogene* 2018;**37**:1369–85. doi:10.1038/s41388-017-0044-8
- 104 Nuvoli B, Sacconi A, Cortese G, *et al.* Reduction of estradiol in human malignant pleural mesothelioma tissues may prevent tumour growth, as implied by in vivo and in-vitro models. *Oncotarget* 2016;**7**:47116–26. doi:10.18632/oncotarget.9964
- 105 Kalra N, Zhang J, Thomas A, *et al.* Mesothelioma patient derived tumor xenografts with defined BAP1 mutations that mimic the molecular characteristics of human malignant mesothelioma. *BMC Cancer* 2015;**15**:1–11. doi:10.1186/s12885-015-1362-2



- 106 Wu L, Allo G, John T, *et al.* Patient-derived xenograft establishment from human malignant pleural mesothelioma. *Clin Cancer Res* 2017;**23**:1060–7. doi:10.1158/1078-0432.CCR-16-0844
- 107 Hamaidia M, Gazon H, Hoyos C, *et al.* Inhibition of EZH2 methyltransferase decreases immunoediting of mesothelioma cells by autologous macrophages through a PD-1-dependent mechanism. *JCI Insight* 2019;**4**. doi:10.1172/jci.insight.128474
- 108 Jackaman C, Bundell CS, Kinnear BF, *et al.* IL-2 Intratumoral Immunotherapy Enhances CD8 + T Cells That Mediate Destruction of Tumor Cells and Tumor-Associated Vasculature: A Novel Mechanism for IL-2 . *J Immunol* 2003;**171**:5051–63. doi:10.4049/jimmunol.171.10.5051
- 109 Ma S, Chee J, Fear VS, *et al.* Pre-treatment tumor neo-antigen responses in draining lymph nodes are infrequent but predict checkpoint blockade therapy outcome. *Oncoimmunology* 2020;**9**:1684714. doi:10.1080/2162402X.2019.1684714
- 110 Klampatsa A, Leibowitz MS, Sun J, *et al.* Analysis and Augmentation of the Immunologic Bystander Effects of CAR T Cell Therapy in a Syngeneic Mouse Cancer Model. *Mol Ther - Oncolytics* 2020;**18**:360–71. doi:10.1016/j.omto.2020.07.005
- 111 Nader JS, Abadie J, Deshayes S, *et al.* Characterization of increasing stages of invasiveness identifies stromal/cancer cell crosstalk in rat models of mesothelioma. *Oncotarget* 2018;**9**:16311–29. doi:10.18632/oncotarget.24632
- 112 Nader JS, Boissard A, Henry C, *et al.* Cross-species proteomics identifies CAPG and SBP1 as crucial invasiveness biomarkers in rat and human malignant mesothelioma. *Cancers (Basel)* 2020;**12**:1–19. doi:10.3390/cancers12092430
- 113 Roulois D, Deshayes S, Guilly MN, *et al.* Characterization of preneoplastic and neoplastic rat mesothelial cell lines: The involvement of TETs, DNMTs, and 5-hydroxymethylcytosine. *Oncotarget* 2016;**7**:34664–87. doi:10.18632/oncotarget.8970
- 114 Digifico E, Erreni M, Colombo FS, *et al.* Optimization of a Luciferase-Expressing Non-Invasive Intrapleural Model of Malignant Mesothelioma in Immunocompetent Mice. *Cancers (Basel)* 2020;**12**:2136. doi:10.3390/cancers12082136
- 115 Mezzapelle R, Rrapaj E, Gatti E, *et al.* Human malignant mesothelioma is recapitulated in immunocompetent BALB/c mice injected with murine AB cells. *Sci Rep* 2016;**6**. doi:10.1038/srep22850
- 116 Davis MR, Manning LS, Whitaker D, *et al.* Establishment of a murine model of malignant mesothelioma. *Int J Cancer* 1992;**52**:881–6. doi:10.1002/ijc.2910520609
- 117 Barbone D, Cheung P, Battula S, *et al.* Vorinostat Eliminates Multicellular Resistance of Mesothelioma 3D Spheroids via Restoration of Noxa Expression. *PLoS One* 2012;**7**. doi:10.1371/journal.pone.0052753
- 118 Barbone D, Follo C, Echeverry N, *et al.* Autophagy correlates with the therapeutic responsiveness of Malignant pleural mesothelioma in 3D models. *PLoS One* 2015;**10**:1–23. doi:10.1371/journal.pone.0134825
- 119 Papazoglou ED, Jagirdar RM, Kouliou OA, *et al.* In vitro characterization of cisplatin and pemetrexed effects in malignant pleural mesothelioma 3d culture phenotypes. *Cancers (Basel)* 2019;**11**. doi:10.3390/cancers11101446
- 120 Kim KU, Wilson SM, Abayasiriwardana KS, *et al.* A novel in vitro model of human mesothelioma for studying tumor biology and apoptotic resistance. *Am J Respir Cell Mol Biol* 2005;**33**:541–8. doi:10.1165/rcmb.2004-0355OC

- 121 Barbone D, Yang TM, Morgan JR, *et al.* Mammalian target of rapamycin contributes to the acquired apoptotic resistance of human mesothelioma multicellular spheroids. *J Biol Chem* 2008;**283**:13021–30. doi:10.1074/jbc.M709698200
- 122 Gerogianni I, Pitaraki E, Jagirdar RM, *et al.* 2-Deoxy-glucose Enhances the Effect of Cisplatin and Pemetrexed in Reducing Malignant Pleural Mesothelioma Cell Proliferation but Not Spheroid Growth. *Anticancer Res* 2019;**39**:3809–14. doi:10.21873/anticancer.13530
- 123 Barbone D, Ryan JA, Kolhatkar N, *et al.* The Bcl-2 repertoire of mesothelioma spheroids underlies acquired apoptotic multicellular resistance. *Cell Death Dis* 2011;**2**:e174-9. doi:10.1038/cddis.2011.58
- 124 Wilson SM, Barbone D, Yang TM, *et al.* mTOR mediates survival signals in malignant mesothelioma grown as tumor fragment spheroids. *Am J Respir Cell Mol Biol* 2008;**39**:576–83. doi:10.1165/rcmb.2007-0460OC
- 125 Kim H, Phung Y, Ho M. Changes in global gene expression associated with 3D structure of tumors: An ex vivo matrix-free mesothelioma spheroid model. *PLoS One* 2012;**7**. doi:10.1371/journal.pone.0039556
- 126 Barbone D, Van Dam L, Follo C, *et al.* Analysis of gene expression in 3D spheroids highlights a survival role for ASS1 in mesothelioma. *PLoS One* 2016;**11**:1–17. doi:10.1371/journal.pone.0150044
- 127 Ruppen J, Cortes-Dericks L, Marconi E, *et al.* A microfluidic platform for chemoresistive testing of multicellular pleural cancer spheroids. *Lab Chip* 2014;**14**:1198–205. doi:10.1039/c3lc51093j
- 128 Mazzocchi AR, Rajan SAP, Votanopoulos KI, *et al.* In vitro patient-derived 3D mesothelioma tumor organoids facilitate patient-centric therapeutic screening. *Sci Rep* 2018;**8**:1–12. doi:10.1038/s41598-018-21200-8
- 129 Sato T, Stange DE, Ferrante M, *et al.* Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 2011;**141**:1762–72. doi:10.1053/j.gastro.2011.07.050
- 130 Van De Wetering M, Francies HE, Francis JM, *et al.* Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 2015;**161**:933–45. doi:10.1016/j.cell.2015.03.053
- 131 Boj SF, Hwang C II, Baker LA, *et al.* Organoid models of human and mouse ductal pancreatic cancer. *Cell* 2015;**160**:324–38. doi:10.1016/j.cell.2014.12.021
- 132 Sachs N, de Ligt J, Kopper O, *et al.* A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. *Cell* 2018;**172**:373-386.e10. doi:10.1016/j.cell.2017.11.010
- 133 Huch M, Gehart H, Van Boxtel R, *et al.* Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* 2015;**160**:299–312. doi:10.1016/j.cell.2014.11.050
- 134 Sachs N, Papaspyropoulos A, Zomer-van Ommen DD, *et al.* Long-term expanding human airway organoids for disease modeling. *EMBO J* 2019;**38**:1–20. doi:10.15252/embj.2018100300
- 135 Kopper O, de Witte CJ, Löhmußaar K, *et al.* An organoid platform for ovarian cancer captures intra- and interpatient heterogeneity. *Nat Med* 2019;**25**:838–49. doi:10.1038/s41591-019-0422-6

- 136 Dijkstra KK, Monkhorst K, Schipper LJ, *et al.* Challenges in Establishing Pure Lung Cancer Organoids Limit Their Utility for Personalized Medicine. *Cell Rep* 2020;**31**. doi:10.1016/j.celrep.2020.107588
- 137 Turco MY, Gardner L, Hughes J, *et al.* Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Nat Cell Biol* 2017;**19**:568–77. doi:10.1038/ncb3516
- 138 Gao D, Vela I, Sboner A, *et al.* Organoid cultures derived from patients with advanced prostate cancer. *Cell* 2014;**159**:176–87. doi:10.1016/j.cell.2014.08.016
- 139 Broutier L, Mastrogiovanni G, Verstegen MMA, *et al.* Europe PMC Funders Group Human Primary Liver Cancer -derived Organoid Cultures for disease modelling and drug screening. 2018;**23**:1424–35. doi:10.1038/nm.4438.Human
- 140 Vlachogiannis G, Hedayat S, Vatsiou A, *et al.* gastrointestinal cancers. 2018;**926**:920–6.
- 141 Ooft SN, Weeber F, Dijkstra KK, *et al.* Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. *Sci Transl Med* 2019;**11**:1–10. doi:10.1126/scitranslmed.aay2574
- 142 Sachs N, Papaspyropoulos A, Zomer-van Ommen DD, *et al.* Long-term expanding human airway organoids for disease modeling. *EMBO J* 2019;**38**:1–20. doi:10.15252/emj.2018100300
- 143 Dijkstra KK, Cattaneo CM, Weeber F, *et al.* Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. *Cell* 2018;**174**:1586-1598.e12. doi:10.1016/j.cell.2018.07.009
- 144 Tsai S, McOlash L, Palen K, *et al.* Development of primary human pancreatic cancer organoids, matched stromal and immune cells and 3D tumor microenvironment models. *BMC Cancer* 2018;**18**:1–13. doi:10.1186/s12885-018-4238-4
- 145 Richards Z, McCray T, Marsili J, *et al.* Prostate Stroma Increases the Viability and Maintains the Branching Phenotype of Human Prostate Organoids. *iScience* 2019;**12**:304–17. doi:10.1016/j.isci.2019.01.028
- 146 Neal JT, Li X, Zhu J, *et al.* Organoid Modeling of the Tumor Immune Microenvironment. *Cell* 2018;**175**:1972-1988.e16. doi:10.1016/j.cell.2018.11.021

Supplementary Table 1. List of human pleural mesothelioma cell lines with characterised histological and genetic background.

Cell line	Origin	Histology/Morphology	Genetic alterations	Ref
MM1	tumour tissue/ pleural effusion	sarcomatoid	loss of <i>CDKN2A</i> (7/9); <i>BAP1</i> point mutation (4/9); <i>NF2</i> loss of function (7/9); kataegis (4/9); chromoplexy (8/9)	[49]
MM2	tumour tissue	desmoplastic		
MM3	tumour tissue	epithelioid		
MM4	tumour tissue	biphasic		
MM5	tumour tissue	biphasic		
MM7	tumour tissue	epithelioid		
MM8	tumour tissue	epithelioid		
MM9	tumour tissue	sarcomatoid		
MM10	tumour tissue	epithelioid		
MPM 1	tumour tissue	epithelioid	NA	[60]
MPM 2	tumour tissue	epithelioid		
MPM 3	tumour tissue	epithelioid		
MPM 4	tumour tissue	biphasic		
MPM 5	tumour tissue	biphasic		
MPM 6	tumour tissue	biphasic		
MPM 7	tumour tissue	sarcomatoid		
MPM 8	tumour tissue	sarcomatoid		
MPM 9	tumour tissue	sarcomatoid		
MESO-3T	tumour tissue	epithelioid	heterozygous loss of <i>NF2</i> (5/8), loss of p16 <sup>INK4a</sup> and p14 <sup>ARF</sup> (7/8), structural abnormalities in chr1, 2, 3, 6, 7 and 9	[48]
MESO-7T	tumour tissue	biphasic		
MESO-8T	tumour tissue	epithelioid with sarcomatoid loci		
MESO-9T	tumour tissue	epithelioid		
MESO-12T	tumour tissue	epithelioid		
MESO-14T	tumour tissue	epithelioid		
MESO-17T	tumour tissue	epithelioid		
MESO-27T	tumour tissue	epithelioid		
MM04	pleural biopsy	desmoplastic	chromosomal abnormalities ranging from 41 to 113; monosomy of chr8, 14, 22 (2/5) and 17 (3/5); deletion/translocation in chr1, 5, 6 (epithelioid type) and chr1, 9, 19 (biphasic type); rearrangement of chr1 (5/5) and chr6 (1/5)	[64]
MM05	pleural biopsy	biphasic		
MM12	pleural biopsy	biphasic		
MM13	pleural biopsy	biphasic		
MM1081	pleural biopsy	epithelioid		
PF1038	pleural effusion	unclassified		
HMM-1	pleural effusion	NA	NA	[57]
HMM-3	pleural effusion	NA	NA	
MPM1	pleural effusion	sarcomatoid	hypotetraploid with a modal chr 79	[55]
MPM2	pleural effusion	epithelioid	hypodiploid with a modal chr 40	
MPM3	pleural effusion	biphasic	hyperdiploid with a modal chr 44	
ACC-MESO-1	tumour tissue	epithelioid	<i>NF2</i> mutation (1/4); p16 <sup>INK4a</sup> and p14 <sup>ARF</sup> homologous deletion (4/4)	[65,66]
ACC-MESO-4	tumour tissue	epithelioid		

Y-MESO-8	pleural effusion	biphasic		
Y-MESO-9	pleural effusion	NA		
Y-MESO-12	pleural effusion	NA		
Y-MESO-14	pleural effusion	NA		
Y-MESO-21	pleural effusion	NA		
Y-MESO-22	pleural effusion	NA		
Y-MESO-25	pleural effusion	NA		
Y-MESO-26B	pleural effusion	NA		
Y-MESO-27	pleural effusion	NA		
Y-MESO-28	pleural effusion	NA		
Y-MESO-29	pleural effusion	NA		
Y-MESO-30	pleural effusion	NA		
MM-Z03S	pleural effusion	sarcomatoid	both cell lines included chromosomal losses of 1q34~qter, 4, 9p, 10p, 13, 14, 16q, 18, and 22, as well as a complex structural aberration involving chr17. MMZ03E had gains of 3q11q27 and 5p, while MM-Z03S had gain of 9q and losses of 3q27qter, 11q, and 18	[56]
MM-Z03E		epithelioid		
MES-BR 95	pleural effusion	biphasic	NA	[67]
MES-CM 98	pleural effusion	epithelioid		
MES-CG 98	pleural effusion	biphasic		
MES-CE 96	pleural effusion	biphasic		
MES-GF 99	pleural effusion	biphasic		
MES-MM 98	pleural effusion	sarcomatoid		
MES-OC 99	pleural effusion	sarcomatoid		
MES-PP 89	pleural effusion	sarcomatoid		
JL1	tumour biopsy	epithelioid	chromosomal deletions and translocations; chromosomal trisomies and monosomies	[68]
DM3	tumour biopsy	sarcomatoid		
PJ3	pleural effusion	biphasic		
HMMME	pleural effusion	spindle, round and polygonal shape	aneuploidy	[69]
IST-Mes1	pleural effusion	epithelioid-like	monosomy of chr17 (4/4), chr13 (3/4), chr22 (2/4); deletion/translocation of chr6 (4/4), chr1 (3/4), chr1 (3/4)	[70]
IST-Mes2	pleural effusion	epithelioid-like		
IST-Mes3	pleural effusion	spindle-shaped		
MPP89	pleural effusion	epithelioid-like		
H2596	tumour tissue	sarcomatoid	deletion, inversion and translocation affecting chr1, 3, 6, 9; chromosome numbers ranging from 34 to 90; abnormalities in number of chr3, 4, 5, 9, 11, 14, 15, 22	[50]
H2591	tumour tissue	epithelioid		
H2461	tumour tissue	epithelioid		
HP-1	pleural effusion	biphasic		
HP-2	pleural effusion	epithelioid		
H2373	tumour tissue	sarcomatoid		
H2452	tumour tissue	biphasic		
HP-3	pleural effusion	epithelioid		
H2595	tumour tissue	epithelioid		
ZL5	pleural effusion	biphasic	NA	[51]
ZL34	tumour tissue	sarcomatoid		

ZL55	tumour tissue	epithelioid		
ZL92	pleural effusion	not determined		
SPC78	tumour tissue	sarcomatoid		
SPC111	pleural effusion	biphasic		
SPC212	tumour tissue	biphasic		
JU77	pleural effusion	spindle-shaped	deletion of long arm of chr6 (4/5); chromosome 1 rearrangements	[52]
LO68	pleural effusion	spindle-shaped		
ONE58	pleural effusion	spindle-shaped		
NO36	pleural effusion	stellate-shaped		
DeH136	pleural effusion	pleomorphic		
1/M9K	metastasis	biphasic	deletion of long arm of chr5 (5/6); monosomy of chr9 (4/6), chr13 (3/6) and chr22 (2/6); rearrangement of chr1 (3/6)	[53]
2/M10K	metastasis	biphasic		
3/M14K	tumour tissue	epithelioid		
3/M20	pleural effusion	epithelioid		
3/M19	pleural effusion	epithelioid		
4/M33K	tumour tissue	biphasic		
5/M38K	tumour tissue	biphasic		
6/M22K	tumour tissue	biphasic		
6/M19	pleural effusion	biphasic		
Mero-25	pleura autopsy	biphasic	abnormalities in number of ch1, 3, 6, 9, 12 and 22; loss of chr4	[54]
Mero-48a	pleura autopsy	biphasic		
Mero-72	pleura biopsy	biphasic		
Mero-96	pleura biopsy	sarcomatoid		
Mero-14	pleural effusion	NA		
Mero-41	pleural effusion	NA		
Mero-64	pleural effusion	NA		
Mero-82	pleural effusion	NA		
Mero-83	pleural effusion	NA		
Mero-84	pleural effusion	NA		
Mero-95	pleural effusion	NA		
P31	NA	Epithelioid	NA	[59][61]
VMC6	NA	Epithelioid	NA	
VMC11	NA	Biphasic	NA	
VMC12	NA	Epithelioid	NA	
VMC14	NA	Epithelioid	NA	
VMC20	NA	Epithelioid	NA	
VMC23	NA	Epithelioid	NA	
VMC28	NA	Epithelioid	NA	
VMC31	NA	Epithelioid	NA	
VMC33	NA	Epithelioid	NA	
VMC40	NA	Biphasic	NA	
Meso49	NA	Epithelioid	NA	
Meso53	NA	Biphasic	NA	
Meso62	NA	Sarcomatoid	NA	
SPC111	NA	Biphasic	NA	
SPC212	NA	Biphasic	NA	
M38K	NA	Biphasic	NA	
MM288	NA	Epithelioid	NA	
				[62]

MM317	NA	Epithelioid	NA	
MM404	NA	Epithelioid	NA	
MM473	NA	Epithelioid	NA	
MM487	NA	Biphasic	NA	
MM491	NA	Biphasic	NA	
MM432	NA	Sarcomatoid	NA	
MM472	NA	Sarcomatoid	NA	
H-MESO-1	Tumour	Epithelioid	Pseudotriploid with 68 chromosomes including multiple abnormalities (monosomy, translocations, deletion, trisomy, and tetrasomy)	[63]

